

MELISOPALINOLOGY OF KLANCENG HONEY FROM 4 LOCATIONS IN KLATEN, MAGELANG, AND BOYOLALI DISTRICTS

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ABSTRACT

Klanceng honey is a type of honey produced by stingless bees. The characteristics of klanceng honey are a liquid texture and a more sour or bitter taste. The content of honey is influenced by the honey's food source, which can be seen from the pollen contained in the honey. Identified pollen can be used to indicate the characteristics of the plants that serve as bee food sources. The purpose of this study was to determine the diversity of pollen sources in klanceng honey from Penggung Village, Boyolali Regency; Kebonrejo Village, Magelang Regency; Ceper Village, and Ngemplak Village, Klaten Regency, which can be used to identify plants that serve as bee food sources and be used to label honey based on its food source. The sampling method used exploratory sampling with purposive sampling. The pollen analysis method used acetolysis. Data analysis used descriptive analysis. The results of melisopalinalogy showed that there were 26 plant pollen families contained in the four klanceng honeys, with coconut (*Cocos nucifera*) pollen being the most dominant. Klanceng honey from Ceper and Kebonrejo villages is monofloral, while honey from Penggung village is bifloral, and honey from Ngemplak village is multifloral. The conclusion of this study is that the plant diversity of klanceng bees' food sources is diverse.

Keywords: klanceng honey, melisapalinology, pollen, food source

INTRODUCTION

Honey is a healthy food produced by bees from sources such as flower nectar (floral nectar), secretions from parts of plants other than flowers (extra-floral plant secretions), or insect excretions (SNI, 2004). Honey contains at least 181 components, dominated by sugars, particularly fructose and glucose, with water content reaching 17%, and other components (Testa et al., 2019). Klanceng honey is honey produced by stingless bees, which usually has a more sour or bitter taste and a liquid texture. Compared to *Apis mellifera* honey, stingless bee honey has higher moisture content, lower diastase activity, and significantly higher electrical conductivity and acidity (Chuttong et al., 2015; Hrnecir et al., 2016).

The components contained in honey are influenced by the food taken by bees, such as pollen and nectar found in plants (Wright, et al., 2020). Pollen and nectar are known to be the main food sources for honey bees, followed by water and plant resin. Nectar is the main source of sugar, and pollen is the main source of protein for bee colonies. Pollen plays an important role in larval growth and colony health (Melin et al., 2020; Wright et al., 2018). The pollen sources chosen by honey bees are not always based on protein content but on plant abundance (Liolios et al., 2016) and the flowering period of plants around the bee hive (Balderas, 2016). This shows that the condition of plants around the hive will influence the bees' preferences in searching for pollen sources (Basari et al., 2021; Wright et al., 2018).

The type of flowers preferred by bees can be determined by identifying the pollen contained in honey (Selvaraju et al., 2019). Each pollen has different characteristics according to the plant. The identification of pollen in honey is called melissapalinology, which involves determining a diagrammatic description of the pollen morphology. The results of melissapalinology analysis can provide information about the characteristics of the plants that are the source of bee food, which can be used to classify bees into multifloral, bifloral, and monofloral honey (Ponuchammy et al., 2014; da Luz et al., 2020). This can be used as a reference for beekeepers in providing ideal conditions around the bee hive, as well as for labeling the honey produced.

METHODS

Place and Time

The research began with honey sampling in Ceper Village, Ceper District; Ngemplak Village, Karangnongko District, Klaten Regency; Kebonrejo Village, Candimulyo District, Magelang Regency; and Penggung Village, Boyolali District, Boyolali Regency. The subsequent research was conducted in the Plant Structure and Function Biology Laboratory, Department of Biology, Faculty of Science and Mathematics. The research was conducted from August 2021 to August 2022.

Tools and materials

The tools used were analytical balances, test tubes, centrifuges, micropipettes, photomicrographs, sample bottles, object glasses, cover glasses, and dropper pipettes. The materials used were klanceng honey samples, methanol solution, sodium hydroxide solution, sulfuric acid (H₂SO₄) solution, acetic anhydride solution, glacial acetic acid (CH₃COOH) solution, and distilled water.

Research Methods

Sampling Methods

Honey sampling used purposive sampling based on differences in farm locations, conducted in four villages, namely Ceper Village, Ceper District; Ngemplak Village, Karangnongko District, Klaten Regency; Kebonrejo Village, Candimulyo District, Magelang Regency; and Penggung Village, Boyolali District, Boyolali Regency. Honey was collected in quantities of 100-300 mL from each cultivation location. Each sample collection was followed by 5 repetitions taken based on differences in glodog.

Vegetation Analysis

Vegetation analysis was used to observe the condition of plants in the vicinity of the bee hives. Vegetation analysis used the quadrat method in accordance with plant habit, namely 2x2 m² observation plots for herbaceous plants; 5x5 m² for shrubs; 10x10 m² for trees with a trunk diameter <20 cm, and 20x20 m² observation plots for trees with a trunk diameter >20 cm. The parameters calculated were density, relative density, frequency, relative frequency, and Importance Value Index for each type of vegetation using the following formulas:

- a. $\text{Kerapatan} = \frac{\text{jumlah individu}}{\text{luas petak}}$
- b. $\text{Kerapatan relatif} = \frac{\text{kerapatan suatu jenis}}{\text{kerapatan seluruh jenis}} \times 100\%$
- c. $\text{Frekuensi} = \frac{\text{jumlah petak terisi suatu jenis}}{\text{jumlah seluruh jenis}}$

$$d. \text{ Frekuensi relatif} = \frac{\text{frekuensi suatu jenis}}{\text{frekuensi seluruh jenis}} \times 100\%$$

$$e. \text{ Indeks Nilai Penting (INP)} = \text{KR}\% + \text{FR}\%$$

Melissopalynological analysis

Melissopalynological analysis used the acetolysis method employed by Majid et al. (2020), with the following steps: 10 g of honey was dissolved in 20 ml of distilled water, dissolved and then centrifuged for 10 minutes at a speed of 2,500 rpm. The supernatant is discarded, and the remaining pellet is added to 3 ml of glacial acetic acid (CH₃COOH), then left to stand for 5 minutes, and centrifuged again for 5 minutes at a speed of 2,500 rpm. The supernatant is discarded, the remaining pellet is washed with 10 ml of distilled water, and centrifuged for 5 minutes at a speed of 2,500 rpm. Washing is repeated until the pH is stable (pH 7) by checking with litmus paper. The pellet with a stable pH is then added with 1 ml of 10% potassium hydroxide (KOH), then heated in a water bath for 5 minutes at a temperature of 70°C. The supernatant is discarded, and ±200 µL of the residue containing pollen is taken and placed on a glass slide, added to the slide, then covered with a cover slip. The preparation is observed under a photomicrograph, and pollen characterization is performed based on its morphology and pollen count.

Pollen counting is performed on samples containing a minimum of 300 pollen grains in each preparation. The results of the calculation will show the frequency (%) of each plant pollen taxon contained in the honey. The frequency (%) value of pollen taxa will be used to determine the pollen taxa frequency type among 4 groups, namely predominant pollen (PP, >45%), secondary pollen (SC, 16-45%), important pollen (IP, 3-15%) and minor pollen (MP, <3%) (Majid et al., 2020). The pollen frequency type will determine the honey group (Wingenroth, 2001).

RESULTS AND DISCUSSION

The honey used in this study was sourced from stingless bee farms in Ceper Village, Ceper Subdistrict; Ngemplak Village, Karangnongko Subdistrict, Klaten Regency; Penggung Village, Boyolali Subdistrict, Boyolali Regency; and Kebonrejo Village, Candimulyo Subdistrict, Magelang Regency. The four bee farming locations have quite different environmental characteristics. Bee farming in Ceper Village is located in a densely populated residential area near a highway, with an altitude of ±118.4 meters above sea level. The bee farming location in Ngemplak Village is in a village surrounded by agricultural land (chili and tomato crops) and plantations (mahogany, sengon, and coconut trees), with an altitude of ± 383 meters above sea level. Bee farming in Kebonrejo Village is surrounded by coconut plantations, with an elevation of ± 448.2 meters above sea level. The bee farming location in Penggung Village is located in a village surrounded by orchards such as mango, durian, passion fruit, coconut, bamboo, and guava trees, with an elevation of ± 567.4 meters above sea level. Different environmental characteristics may affect the condition of the honey produced.

Vegetation Analysis

Vegetation analysis is used to determine the structure and composition of vegetation in a plant community (Sari, et al., 2018). The vegetation counted includes herbaceous, shrub, and tree plants. Each habitus will have an Importance Value Index (INP). The INP value serves as a measure to show the position of a plant species relative to other species in a community. The higher the INP value of a plant, the more important its role in the community (Shabirin et al., 2020).

The results of vegetation analysis from the four nest locations showed 93 plant species. Several plant species were found in almost all bee nest locations, such as *Amaranthus* sp. and *Cocos nucifera*, with fairly high INP values. *Amaranthus* sp. is a plant that can grow wild in various locations and environmental conditions due to its high adaptability to environmental conditions (Huang, et al., 2019). The data presented shows the plant species of each habitus at each nest location with the highest INP value.

No	Lokasi Sarang	Habitus (INP)			
		Herba	Perdu	Pohon <20 cm	Pohon >20 cm
1.	Desa	<i>Amaranthus</i> sp.	<i>Jatropha curcas</i>	<i>A. heterophyllus</i>	<i>A. heteophyllus</i> (6,07%)
	Ceper	(42,85%)	(4,85%)	(6,12%)	<i>Ficus benjamina</i> (6,07%)
2.	Desa Ngemplak	<i>Amaranthus</i> sp.	<i>S. mahagoni</i> (3,32%)	<i>S. mahagoni</i>	<i>C. nucifera</i> (2,71%)
		(26,44%)		(2,86%)	
			<i>C. nucifera</i> (2,88%)	<i>C. nucifera</i>	<i>S. mahagoni</i> (2,69%)
		<i>Cyperaceae</i>		(2,68%)	
3.	Desa Kebonrejo		<i>G. gnemon</i> (2,62%)	<i>G. gnemon</i>	<i>G. gnemon</i> (2,59%)
				(2,60%)	
		<i>Amaranthus</i> sp.	<i>Curcuma longan</i>	<i>Musa</i> sp. (4,17%)	<i>C. nucifera</i> (3,46%)
		(25,02%)	(5,9%)		
4.	Desa Penggung	<i>Selaginella</i> sp.	<i>Calliandra</i> sp.	<i>C. nucifera</i>	<i>Durio</i> sp. (3,44%)
		(25,02%)	(4,92%)	(3,96%)	
			<i>Coffea</i> sp. (3,58%)	<i>Durio</i> sp. (3,64%)	<i>Albizia</i> sp. (3,42%)
5.	Desa Penggung	<i>Amaranthus</i> sp.	<i>Coffea</i> sp. (3,8%)	<i>Coffea</i> sp.	<i>C. nucifera</i> (3,21%)
		(44,13%)		(3,26%)	
				<i>S. mirtifolium</i>	
		<i>Caliptocarpus</i> sp.	<i>C. nucifera</i> (3,71%)	(3,19%)	<i>Albizia</i> sp. (3,19%)
6.	Desa Penggung			<i>Albizia</i>	
				sp. (3,19%)	

The vegetation found around the hive location in Ceper Village consists mostly of plants deliberately planted by the owners, as the hive is located behind houses in a fairly densely populated village. However, some plants are wild plants that grow on their own, such as *Amaranthus* sp., *Jatropha* sp., *Murayya* sp., and *Morus* sp. The vegetation found around the nest site includes ornamental plants, such as *Sansiviera* sp., *Euphorbia* sp., *Clitoria* sp., *Phalaenopsis* sp., and *Aglonema* sp. Tree-like plants, such as *Muntingia calabura*, *Carica papaya*, *Psidium guajava*, and *Dimocarpus longan*, found around the site are almost all planted by the owner. These plants are utilized for their fruits, and their flowers are used as a food source for bees.

C. nucifera, *G. gnemon*, and *S. mahagoni* plants have the highest INP values in the three observation plots at the bee cultivation site in Ngemplak Village. These three plants have useful benefits for the owners, namely the fruits of *C. nucifera* and *G. gnemon*, and the wood of *S. mahagoni* (Personal communication, 2023). The highest INP value for the herbaceous habit observation plot was for the *Amaranthus* sp. plant, with a value of 26.44%, followed by the *Cyperaceae* plant (23.70%). Both plants are wild plants that have a wide adaptability to environmental conditions.

The location of the stingless bee hives in Kebonrejo Village is in a *C. nucifera* plantation, so *C. nucifera* plants were found in the observation plot for tree habitus with a diameter of < 20 cm and > 20 cm. The vegetation analysis results showed that *C. nucifera* plants had the highest INP value in the observation plot of trees with a diameter > 20 cm, namely 3.46%. This was because *C. nucifera* plants were dominated by mature plants that had been used by the community for many years.

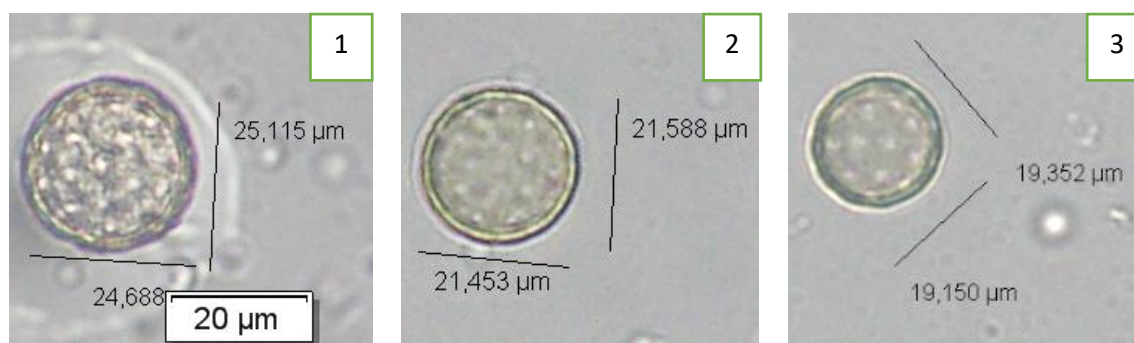
The results of the herbaceous vegetation analysis in Penggung Village showed that many plants grew wild, with the highest INP value in *Amaranthus* sp. plants, namely 44.13%. The highest INP value in the shrub observation plot was in *Coffea* sp. plants (3.8%), followed by *C. nucifera* plants (3.71%). This is because the owners cultivate *C. nucifera* plants from fruit independently in the land behind their houses, adjacent to the bee hives. The results of the tree-level vegetation analysis show that many cultivated plants are used for their fruit. The highest INP value in tree vegetation with a diameter of < 20 cm is *Coffea* sp, while in trees with a diameter of > 20 cm it is *C. nucifera*.

Melissopalynology Analysis

Melissopalynology is a microscopic test to identify pollen found in honey (Bodor et al., 2021). Pollen can be used to determine the composition of the plants that bees feed on, because pollen morphology is not damaged after passing through the bee's digestive process. This is because the outer wall structure of pollen (exine) is composed of sporopollenin, which is strong and resistant to digestive enzymes in bees (Aylanc, et al., 2021). The pollen contained in honey indicates the diversity of plants that are the source of bee feed (Louveaux et al., 1978).

Melissopalynological analysis of honey samples from Ngemplak Village, Ceper Village, Penggung Village, and Kebonrejo Village showed that there were 75 types of plant pollen that served as food sources for bees. There were 18 types of pollen identified at the family level, while 47 types of pollen were identified at the genus level. This shows that the food sources for bees in the four cultivation locations are very diverse.

The plant family that dominates in terms of pollen quantity is Arecaceae, with *Cocos nucifera* pollen being the most abundant in the four types of honey. *C. nucifera* pollen was found in all four types of honey with a frequency value of $>45\%$. This indicates that *C. nucifera* pollen is a type of pollen that is preferred by stingless bees (Majid et al., 2020). *C. nucifera* pollen was found in the nests of three species of stingless bees in Thailand, namely *Tetragonula leaviceps*, *T. testaceitarsis*, and *Lepidotrigona terminata* (Chuttong et al., 2018).



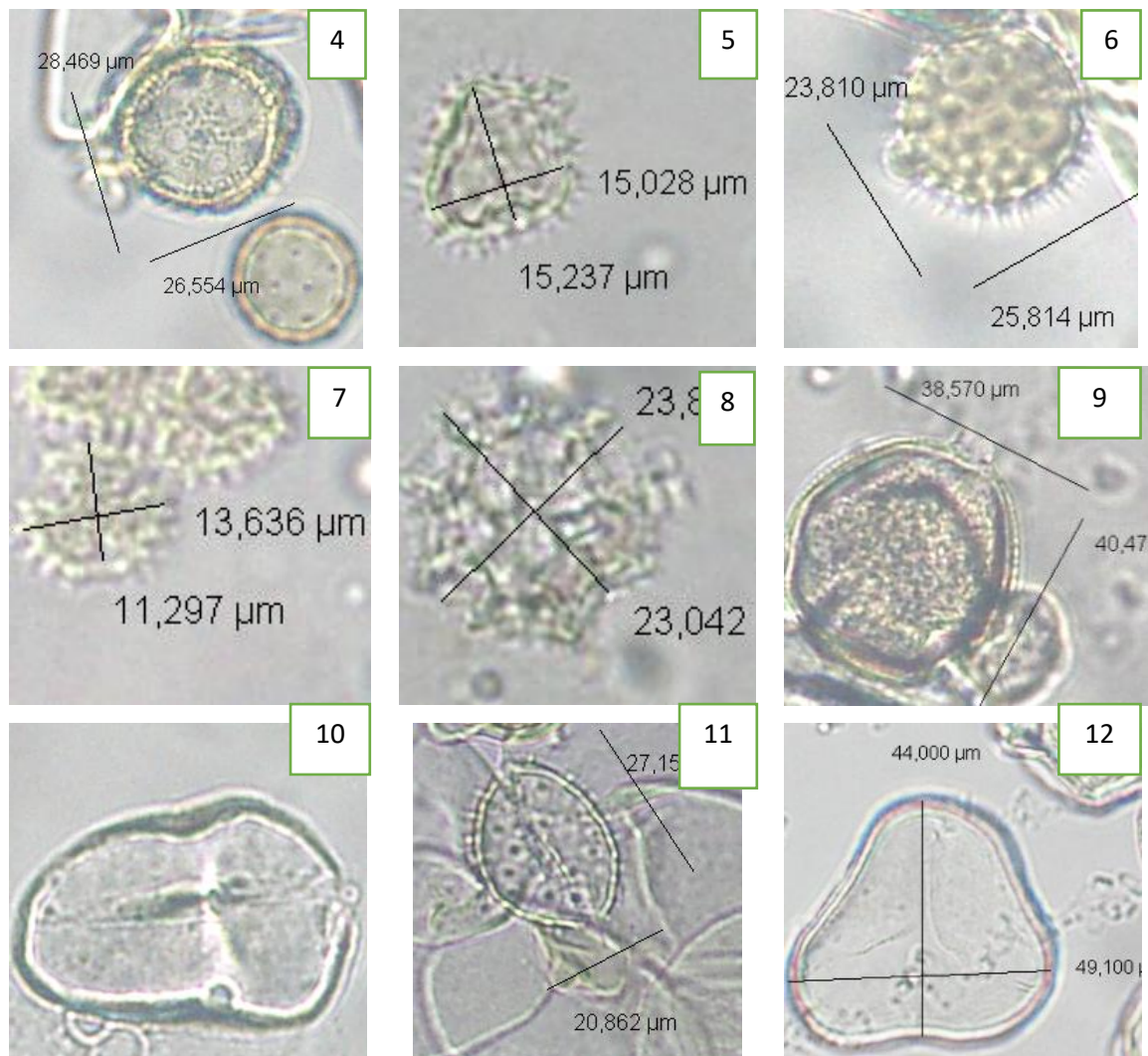
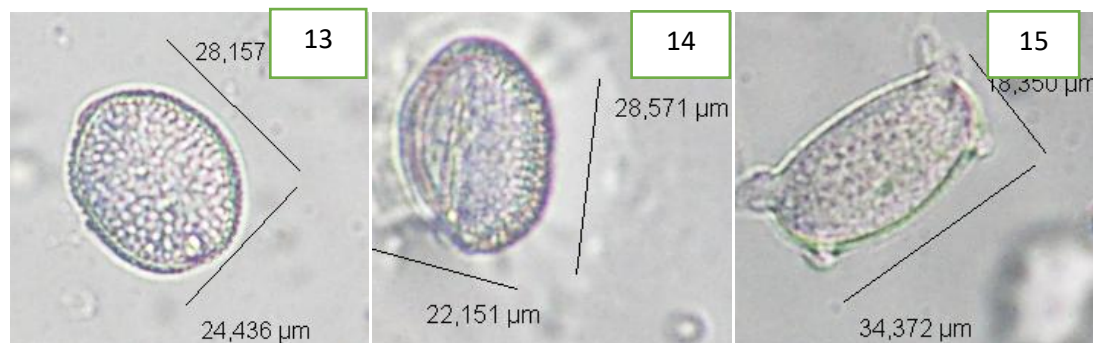


Figure 1. Morphology of pollen from plants that are sources of bee food

Description: 1. *A. tricolor*; 2. *A. dubius*; 3. *A. spinosus*; 4. *Chenopodium album*; 5. *Ageratum conyzoides*; 6. *Biden pilosa*; 7. *Chromolaena odorata*; 8. *Cyanthilium cinerum*; 9. *Mangifera indica*; 10. *Cocos nucifera*; 11. *Arenga pinnata*; 12. *Elaeis sp*



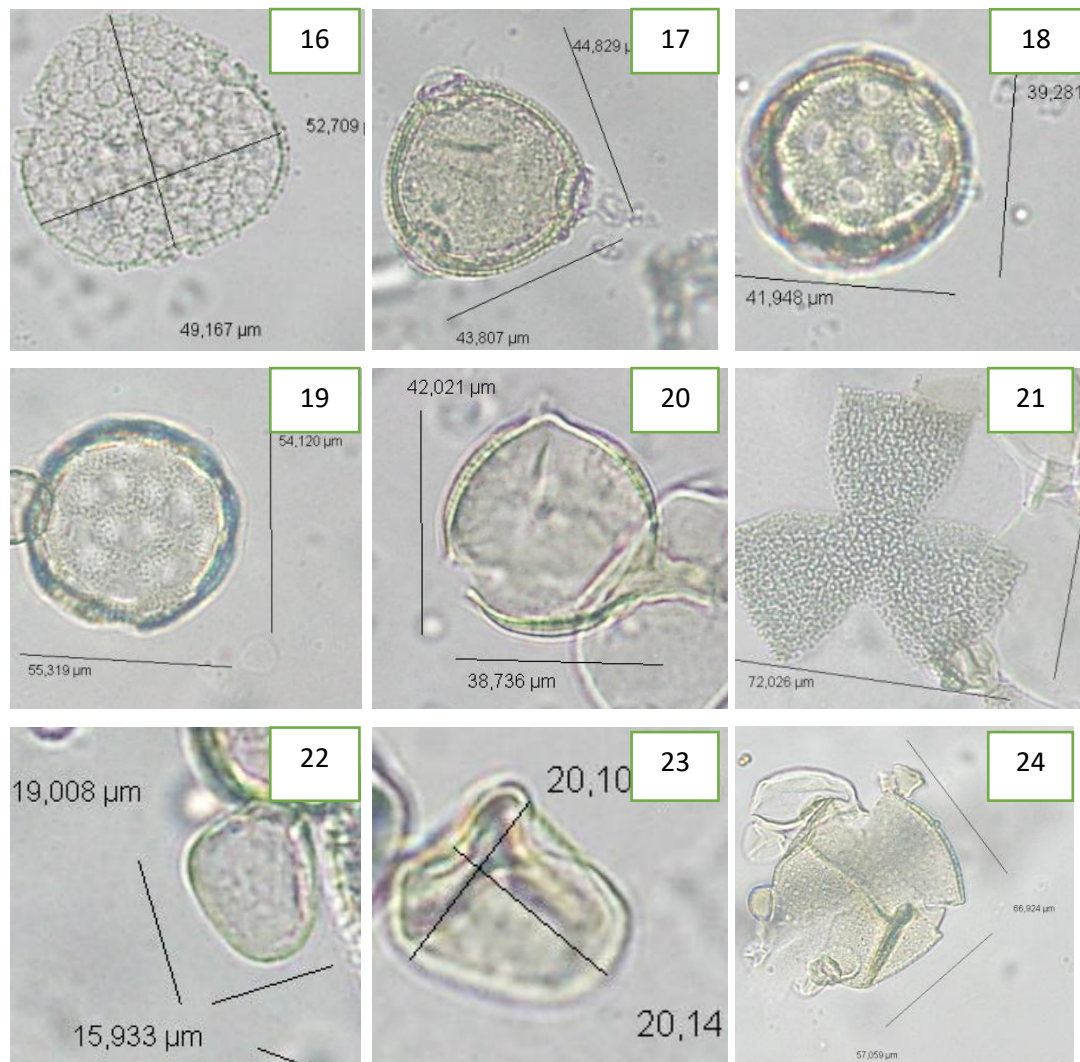
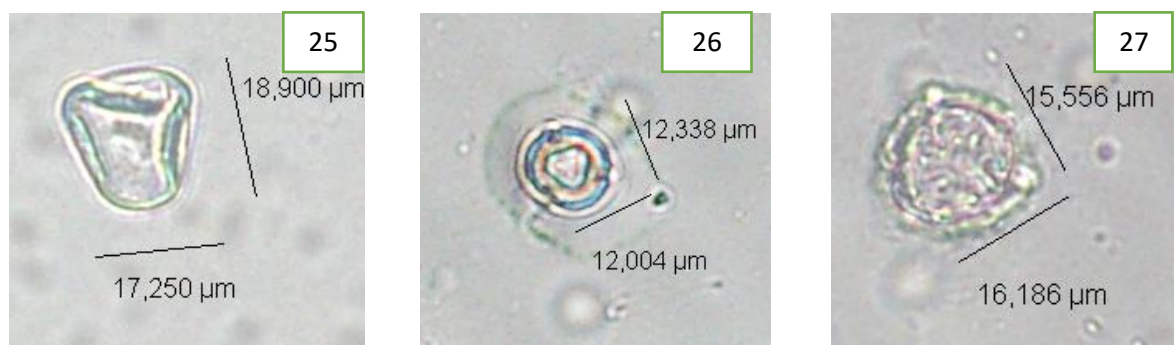


Figure 4. 2. Morphology of pollen from plants that are sources of bee food

Description: 13. *Brassica nigra*; 14. *Raphanus* sp.; 15. *Impatiens* sp.; 16. *Ceiba petandra*; 17. *Cucumis sativus*; 18. *Cerastium holostoides*; 19. *Stellaria* sp.; 20. *Cistus albidus*; 21. *Convolvulus* sp.; 22. *Cyperus pulchellus*; 23. *Cyperus rotundus*; 24. *Cloredendrum* sp.



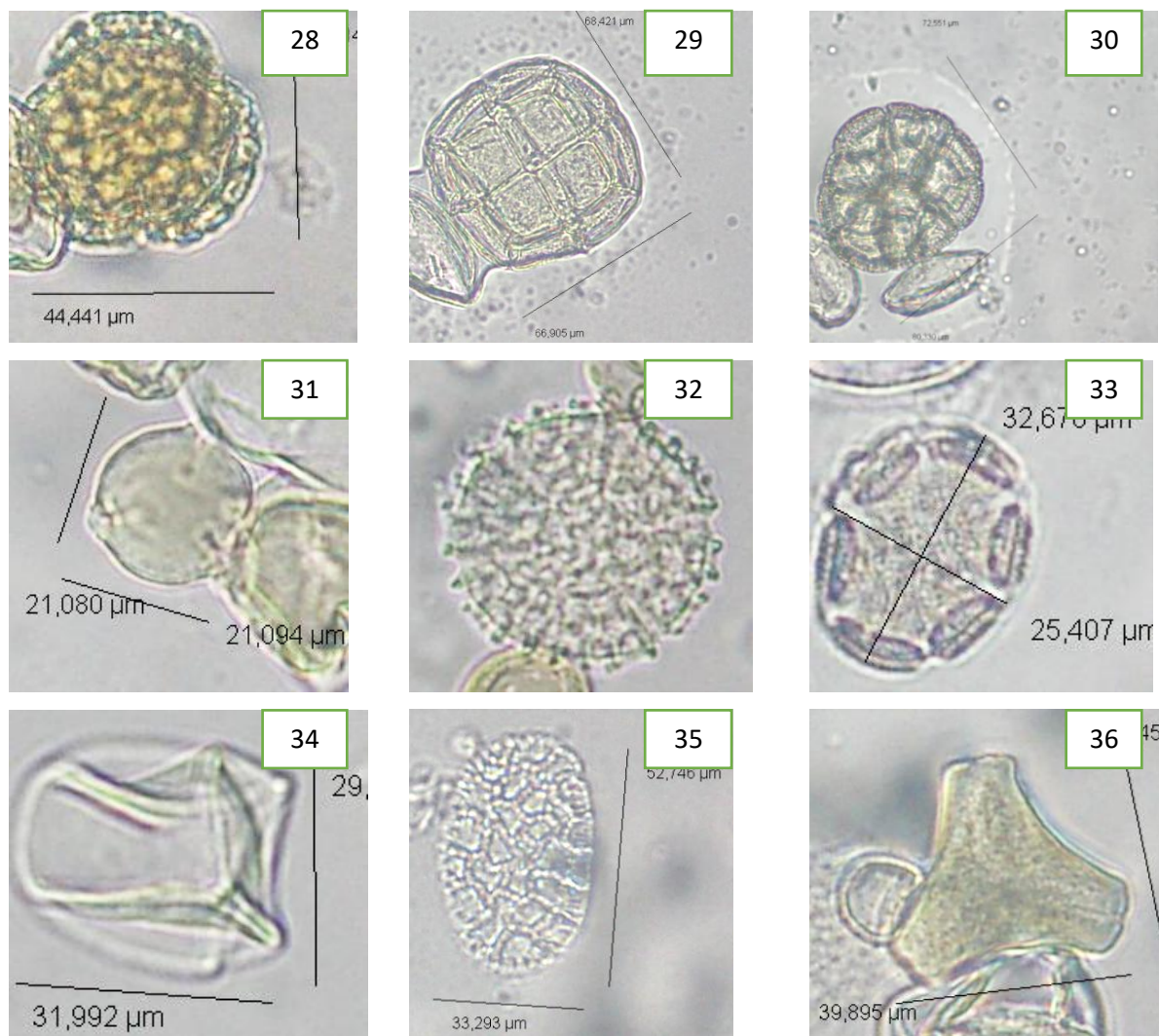
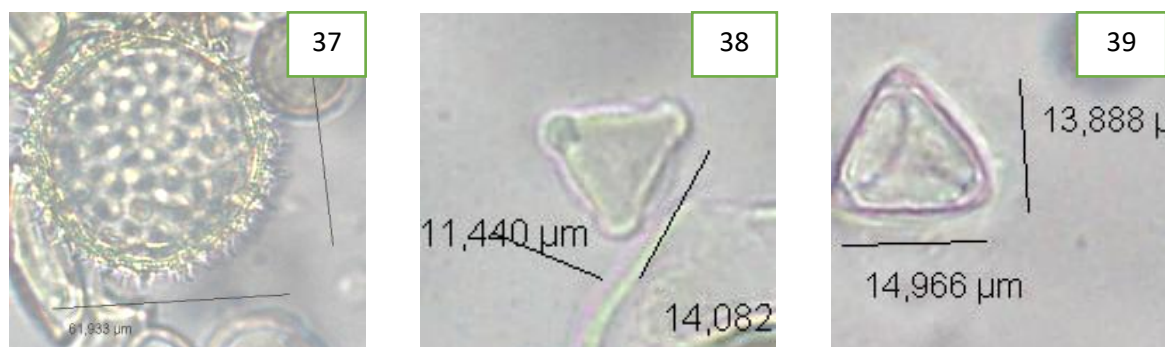


Figure 4. 3. Morphology of pollen from plants that are sources of bee food

Description: 25. *Cyperus* sp.; 26. *Acalypha indica*; 27. *Blumeodendron kurzii*; 28. *Euphorbia heterophylla*; 29. *Acacia* sp.; 30. *Acacia* sp. type 2; 31. *Cassia* sp.; 32. *Ocimum basilicum*; 33. *Hyptis* sp.; 34. *Cyperus ustulatus*; 35. *Lilium* sp.; 36. *Macrosolen cochichinensis*



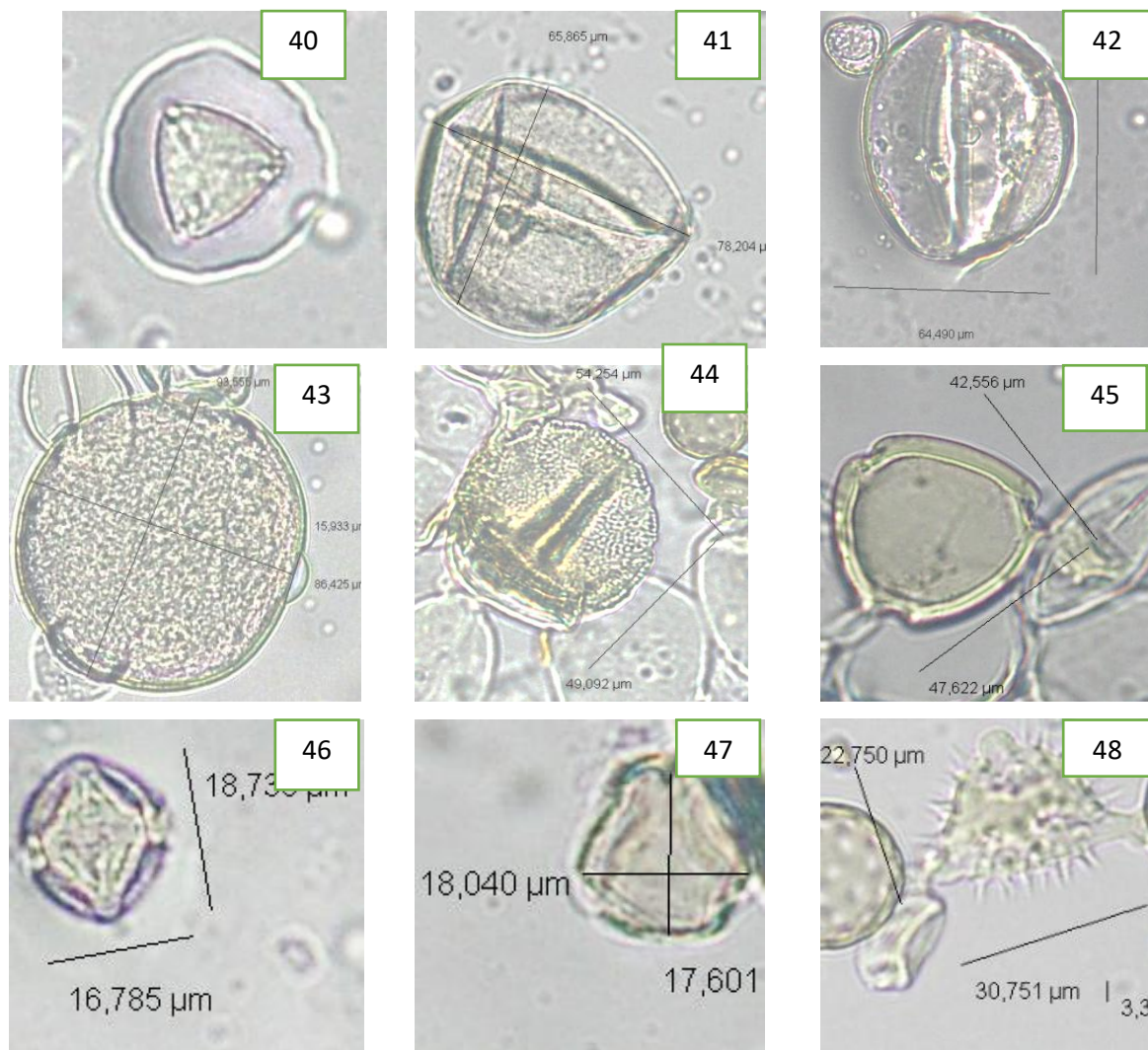
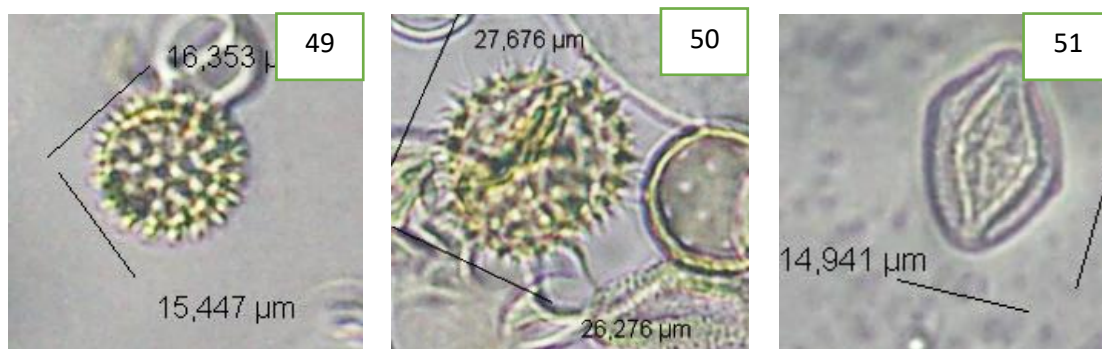


Figure 4. 4. Morphology of pollen from plants that are sources of bee food

Description: 37. *Sida fallax*; 38. *Calistemon salignus*; 39. *Syzygium* sp.; 40. *Psidium guajava*; 41. *Ischaemum rugosum*; 42. *Stipa cipensis*; 43. *Zea mays*; 44. *Polygala* sp.; 45. *Physalis* sp.; 46. *Solanum trifolium*; 47. *Solanum nigrum*; 48. *Asteraceae* type 1.



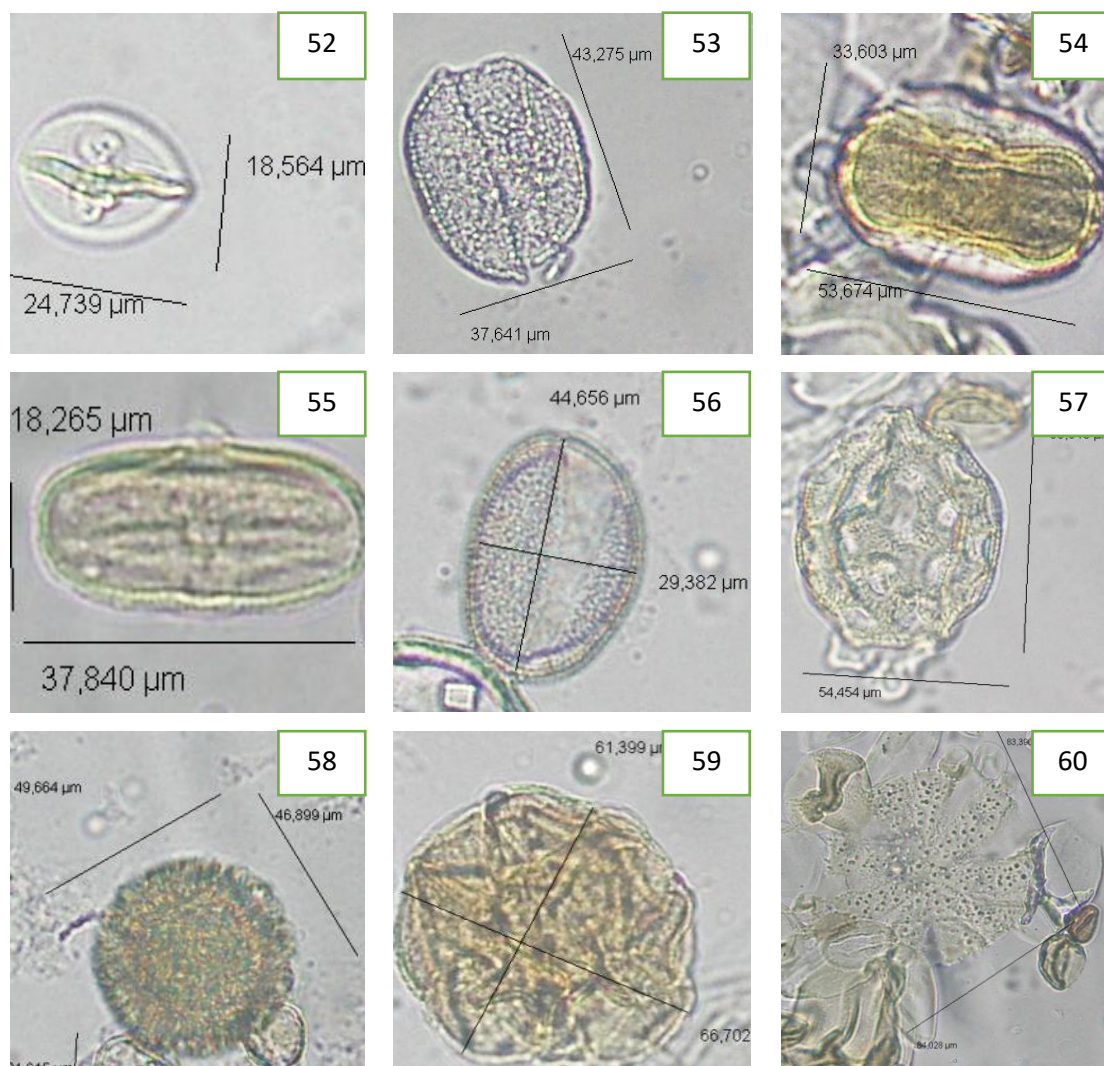


Figure 4. 5. Morphology of pollen from plants that are sources of bee food

Description: 49. Asteraceae type 2; 50. Asteraceae type 3; 51. *Ischaemum* sp.; 52. Anacardiaceae type 1; 53. Arecaceae type 1; 54. Apiaceae type 1; 55. Apiaceae type 2; 56. Bombacaceae type 1; 57. Chenopodiaceae type 1; 58. *Croton* type 1; 59. *Parkia* type 1; 60. Lamiaceae type 1.

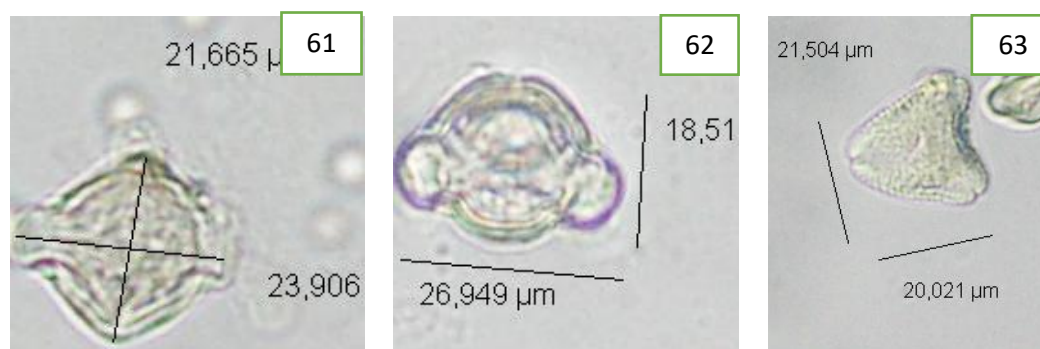


Figure 4. 6. Morphology of pollen from bee forage plants

Description: 61. *Solanum* type 1; *Solanum* type 2; Sapindaceae type 1.

The pollen family with a fairly high frequency after Arecaceae is Amaranthaceae, with the most commonly found pollen being *Amaranthus dubius* in honey from Ngemplak Village, Ceper Village, and Penggung Village. The highest frequency of *A. dubius* pollen in honey from Penggung Village reached 39.81%. This is because the vegetation around the hives in these three villages contains many amaranth plants. The vegetation analysis results showed that spinach plants (*Amaranthus* sp) had a relative density value of > 20% in the four nest locations. According to Riendriasari & Rahayu (2022) and ÖZLER (2018), Amaranthaceae plants are plants that are often used by *Tetragonula leaviceps* stingless bees as a food source, especially a pollen source.

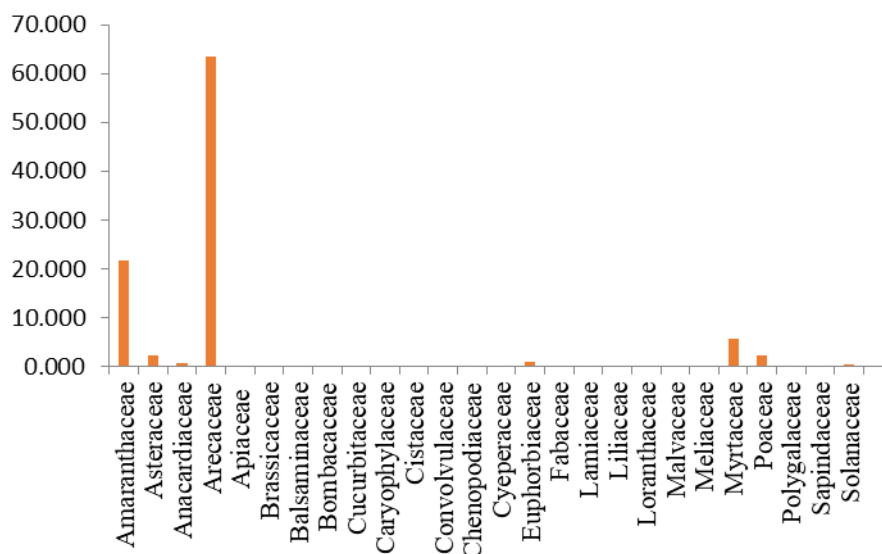


Figure 1. Family-level pollen frequency

Honey samples from Ngemplak Village show that the most dominant pollens are *Cocos nucifera* (45.24%), *Psidium guajava* (18.74%), and *Amaranthus dubius* (16.95%). The abundance of *C. nucifera* and *A. dubius* pollen is supported by vegetation analysis results showing that both plants are available around the hive location. Ngemplak Village klanceng honey is classified as multiflora honey based on Wingenroth's (2011) criteria.

Pollen analysis of Ceper Village honey identified 34 types of pollen. The most dominant pollen was *C. nucifera* with a frequency value of 85.85%, which is classified as predominant pollen. The other 33 types of pollen had a frequency value of <3%, which is classified as minor pollen. This indicates that Ceper Village stingless bee honey is monoflora honey.

The dominance of *C. nucifera* pollen in Ceper Village honey is not supported by the results of vegetation analysis around the nest location, where no coconut trees were found within a distance of 60 m. It is suspected that stingless bees search for food sources further away from the nest due to the low availability of food sources. The hive is located in a densely populated residential area with limited open space. According to Machado et al. (2020), honey bees will search for food sources far from the hive if food sources are scarce in the vicinity of the hive.

Pollen analysis shows that Kebonrejo Village klanceng honey contains 17 plant pollens. *C. nucifera* pollen had the highest frequency value of 97.73%, making it the predominant pollen. The dominance of *C. nucifera* pollen was due to the location of the hive in a coconut plantation. The availability of *C. nucifera* plants in the surrounding environment, which flower

throughout the year, meant that bees tended to use them as their main food source. According to Selvaraju et al. (2019), bees tend to collect pollen and nectar from plants located around the hive, making it more efficient and saving energy for flying.

Pollen analysis results show that the pollen identified in Penggung Village's klanceng honey is the most abundant compared to the other three types of klanceng honey, with 46 plant pollens. The pollen with the highest frequency is *C. nucifera* pollen (47.76%), which is classified as Predominant Pollen, and *A. dubius* pollen (39.81%), which is classified as Secondary Pollen. There were 42 types of pollen with a frequency of 0-3%, consisting of herbaceous, shrub, and tree plants. The pollen frequency value shows that the bee pollen from Penggung Village is biflora honey, as it is dominated by two types of pollen, namely *C. nucifera* and *A. dubius*.

CONCLUSIONS

The vegetation around the cultivation sites in the four villages consists of herbs, shrubs, and trees. The sources of bee food identified from the pollen content in honey from the four cultivation sites are very diverse. The honey from Ceper and Kebonrejo villages is monofloral honey dominated by *Cocos nucifera* pollen. The honey from Penggung village is bifloral honey, and the honey from Ngemplak village is multifloral honey.

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